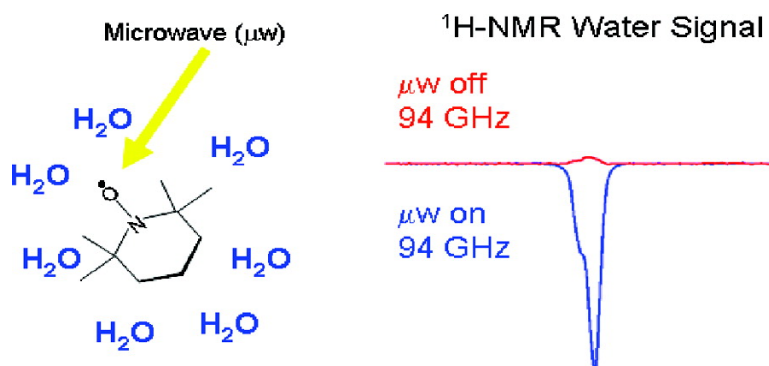


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## Field Dependent Dynamic Nuclear Polarization with Radicals in Aqueous Solution

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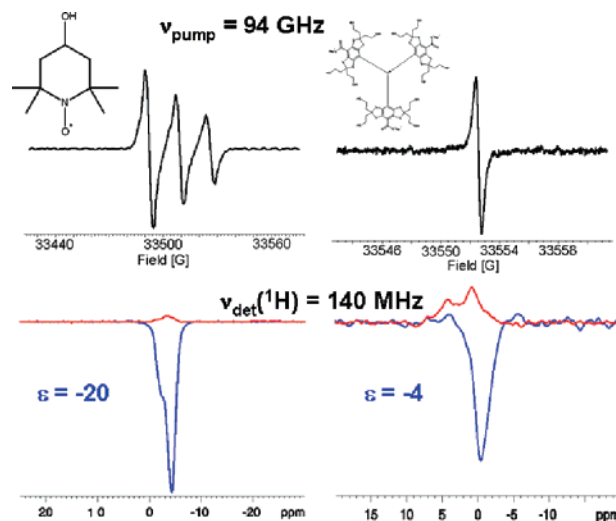
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Dynamic nuclear polarization (DNP) of nuclei in liquid solutions containing paramagnetic centers has been studied since the early years of magnetic resonance to obtain information about molecular motion and electron–nuclear spin relaxation of molecules.<sup>1,2</sup> Previous studies have been performed under low magnetic field strengths ( $B \leq 1$  T) for differing reasons; namely, the Overhauser effect between electrons and nuclei loses efficiency with increasing field strength and the experimental setup for DNP at higher microwave frequencies required technology that was not available to contemporary NMR/EPR laboratories. Recently, DNP has been attracting considerable attention since it can provide a means to overcome the current NMR sensitivity limits that permit the study of macromolecular complexes and doing so under physiologically low concentrations.<sup>3–6</sup> In this context, we have examined the DNP effect in liquids using a state-of-the-art, commercially available microwave technology and a simple DNP polarizer system, consisting of an aqueous solution of the most common nitroxide radical spin probe, suited for biophysical studies. We report <sup>1</sup>H DNP experiments of water solutions containing <sup>14</sup>N-4-hydroxy-TEMPO (TEMPOL) at external magnetic field strengths of 0.34 and 3.3 T that correspond to 9.6 and 94 GHz EPR microwave pumping frequencies, respectively. We observed, for the first time, real nonextrapolated DNP enhancements as large as roughly  $-100$  at 0.34 T and  $-20$  at 3.3 T. Comparison of the TEMPOL data with enhancements by another proposed polarizer, a triarylmethyl-based (TAM or trityl) radical,<sup>7,8</sup> under the same conditions shows that the latter radical is much less well suited for solution state DNP at ambient temperature. The large enhancements observed at low and high fields open up attractive perspectives for potential applications for such polarizers in liquid-state NMR.

DNP in solution is governed by the Overhauser effect,<sup>9</sup> a property by which saturation of the electronic transitions in an electron–nuclear coupled spin system leads to transfer of electron spin polarization, first to the adjacent then subsequently to distal nuclei. The maximum achievable enhancement,  $\epsilon$ , is defined by<sup>1</sup>

$$\epsilon = \frac{\langle I_z \rangle}{I_0} = 1 - \xi f s \frac{|\gamma_s|}{\gamma_I}$$

where  $I_z$  and  $I_0$  are the dynamic and Boltzmann nuclear polarizations, respectively,  $\gamma_s$  and  $\gamma_I$  are the magnetogyric ratios of the electrons and the nuclei,  $f$  is the leakage,  $s$  is the saturation, and  $\xi$  is the coupling factor. The coupling factor,  $\xi$ , depends on the involved relaxation mechanisms and can achieve magnitudes in the range of  $-1$  for pure scalar relaxations and up to  $+0.5$  for pure dipolar relaxations.<sup>1</sup> With the <sup>1</sup>H liquid DNP, the enhancements



**Figure 1.** (Top) EPR lines of TEMPOL and TAM in aqueous solution at 94 GHz,  $T_R$ ,  $c = 5$  mM. The line widths are 2.5 G and 0.3 G at the low-field hyperfine line for TEMPOL and TAM, respectively. (Bottom) <sup>1</sup>H DNP enhancements of water doped with TEMPOL (left) and with TAM (right) at an external field of 3.3 T. Inset: Structure of TEMPOL and of TAM.

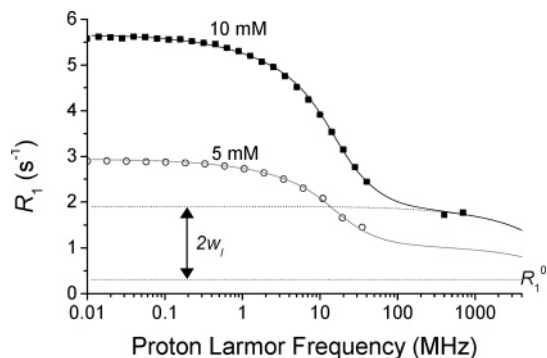
are usually dominated by dipolar relaxation which causes inversion of the nuclear polarizations (negative enhancement) and a maximum possible  $|\epsilon|$  of  $\sim 330$ . The efficiency depends also critically on the saturation factor,  $s$ . Specifically, the pumping (saturation) efficiency is considerably hindered by the microwave attenuation and severe heating effects due to the large dielectric losses in water. Furthermore, the high radical concentrations ( $c > 1$  mM) required in DNP lead to intermolecular spin exchange and to short relaxation times which hamper the saturation effect. This results in a large spread of observed enhancements documented in the literature, particularly with the use of nitroxide radicals.<sup>10–13</sup>

Our experiments were performed using commercial Bruker EPR spectrometers operating at 9.6 and 94 GHz. At 9.6 GHz, a dielectric resonator was used that achieves a  $B_1$  field of 3 MHz at a microwave power,  $P_{\max}$ , of 5 W in CW mode. At 94 GHz, a cylindrical cavity was used in combination with a  $P_{\max}$  of 0.1 W, leading to a  $B_1$  of 8 MHz. Both EPR resonance structures were critically coupled. To permit NMR detection, an ENDOR probe head was connected to a Bruker Minispec ( $\nu(^1\text{H}) \approx 0–60$  MHz) or a Bruker Avance NMR ( $\nu(^1\text{H}) \approx 100–300$  MHz) spectrometer and the ENDOR circuit was externally matched to the NMR receiver. Both types of polarizer sample, TEMPOL and TAM, were dissolved in water at concentrations between 5 and 25 mM. The solutions were degassed and loaded into 1 or 0.15 mm capillary tubes with 8  $\mu\text{L}$  and 45 nL volumes, respectively. Samples were irradiated continuously with

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**Figure 2.**  $^1\text{H}$  water relaxation rates of TEMPOL ( $T = 298\text{ K}$ ) as a function of the applied magnetic field. The straight lines are fits with the model described in the text. The  $2w_1$  component for the 10 mM profile is also shown.

microwaves for 2 s or until the steady state was reached and the subsequent NMR FID was recorded.

Figure 1 illustrates typical  $^1\text{H}$ -DNP enhancements of water doped with TEMPOL and TAM. For TEMPOL, the enhancements reached values up to  $\sim -100$  at 9.6 GHz and  $\sim -20$  at 94 GHz with maximum available power and at concentrations ranging between 5 and 10 mM. With the concentration approaching 25 mM, we observed a decrease in enhancement which was correlated with an increasing EPR line width due to spin exchange effects.<sup>13</sup> To test the quality of our data, we measured additional enhancements with aqueous solutions of the TAM under the same experimental conditions as those for TEMPOL. The latter system is easier to saturate due to its single narrow EPR line at 0.3 G at W-band ( $c = 5\text{ mM}$ ) and had been investigated extensively at  $\sim 300\text{ MHz}$ <sup>7</sup> and  $34\text{ GHz}$ <sup>8</sup> pumping frequencies. With TAM we observed enhancements of  $-40$  at 9.6 GHz and  $-4$  at 94 GHz at the power available. These values lie within the range expected for the frequency dependence reported by Wind et al.<sup>8</sup> and are significantly less than the values we observed with TEMPOL.

There have been ample publications describing the difficulties in evaluating coupling factors with nitroxide radicals. The three nitroxide hyperfine lines cannot be saturated independently since Heisenberg exchange and fast  $^{14}\text{N}$  relaxation redistribute the populations of the electron Zeeman manifolds.<sup>10,11,13,14</sup> Restricted by these parameters, it appeared ambiguous to us whether to extract either a saturation factor or a coupling factor from the DNP data. Instead, we chose to evaluate the coupling factor independently from nuclear magnetic relaxation dispersion measurements (NMRD) as previously proposed in the literature.<sup>1</sup> A similar analysis had been performed previously for the TAM radical.<sup>7,8</sup> The NMRD data of water doped with TEMPOL are displayed in Figure 2. The  $^1\text{H}$  relaxation rates show a linear dependence in concentration as expected for a dipolar relaxation mechanism. The fits were performed using a model for pure dipolar relaxation including free diffusion of the water molecules and fast exchanging protons dipolarly coupled to the radical.<sup>15–17</sup> The fit delivered a correlation time for the dipolar interaction of about 15–20 ps and a closest distance between the water protons and the free electron of about 2.7 Å. When the  $^1\text{H}$  relaxation rate is dominated by a dipolar mechanism, as under these circumstances, the coupling factor can be extracted from the relaxation rate ( $R_1$ ) with the relation<sup>1</sup>

$$\xi = \frac{5}{7} \left\{ 1 - \frac{2w_1}{R_1 - R_1^0} \right\}$$

where  $R_1^0$  is the rate without a paramagnet and  $w_1$  is the rate representing the transition probability between nuclear sublevels. Using the  $R_1$  values from Figure 2 at  $\nu(^1\text{H})$  of 15 and 140 MHz (or 0.34 and 3.4 T, respectively) and  $w^0 = 0.33\text{ s}^{-1}$ , we obtain from the curve at  $c = 10\text{ mM}$  (with  $2w_1 = 1.55\text{ s}^{-1}$ )  $\xi = 0.36 \pm 0.02$  at 9.6 GHz and  $0.06 \pm 0.02$  at 94 GHz. It should be noted that  $\xi$  is not concentration dependent. With knowledge of the leakage factor  $f = (1 - R_1^0/R_1)$  from the NMR data, we were then able to calculate from eq 1 an effective saturation factor of  $0.46 \pm 0.02$  at 9.6 GHz and  $0.75 \pm 0.25$  at 94 GHz. The latter value is larger likely due to the higher microwave pumping field of 94 GHz vs 9 GHz. The fact that the effective saturation factor is higher than  $s = 1/3$ , although pumping only one of the three hyperfine lines, is due to the fast  $^{14}\text{N}$  nuclear relaxation indirectly saturating the neighboring hyperfine lines.<sup>13,14</sup> Our results predict that the observed DNP effect, at both low and high fields, could be much larger if a saturation factor of 1 could be reached.

In conclusion, our results give evidence that TEMPOL in water is a well-suited polarizer system for DNP experiments in aqueous solution. We attribute the improved DNP performance of TEMPOL, with respect to the TAM, to the smaller molecular size which leads to a faster correlation time governing the DNP effect (15–20 ps for TEMPOL vs 142 ps for the TAM<sup>8</sup>). The large enhancements at low and high fields could be employed to increase the sensitivity of solution state NMR experiments if the high polarization of the water protons could be transferred to target biomolecules. The observed enhancement of  $-20$  at 94 GHz would constitute an effective factor of at least  $-4$  when transferring the sample into a 600 MHz NMR spectrometer, assuming other losses were not incurred. Work is currently in progress toward these ends to prove the concept.

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